IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent Application

TAKESHITA et al.

Application Number: 10/812,895

Filed: March 31, 2004

For: APPARATUS FOR MICROINJECTION OF SAMPLE, INTO AMPHIBIAN OOCYTES

Art Unit 1632

Examiner
Parsa Jr., Peter

Attorney Docket No. HIRA.0147

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF ONE SKILLED IN THE ART UNDER 37 C.F.R. 1.132

Sir:

I, Jun DTDMO, am a co-inventor of the above identified application, and hereby declare as follows:

I have reviewed the above-referenced patent application and carefully considered the Examiner's rejection based upon US Patent No. 5,643,938 to Brown (hereinafter "Brown"). It is my conclusion that the invention achieved the "unexpected results" of at least providing high and uniform expression efficiency as discussed as follows, which were not intended, taught, or suggested by Brown. Specifically, it is my opinion that someone of skill in the art would not be motivated to inject mRNA into a plurality of oocytes at "an identical depth" from a surface of each of the oocytes in view of Brown.

The feature of the present invention is a plurality of amphibian oocytes into which a sample introducing mRNA is injected at an identical depth in the range of 0.02-0.1 mm.

As shown in the reference Fig. 1, the oocytes have been injected a sample including DNA at the identical depth in the range of 0.02-0.5 mm to have a high expression efficiency which is useful for screening (p. 9, lines 24-27; p.13, lines 7-10 of the specification). In particular, Applicants discovered that the depth of 0.2 mm is the maximum injection depth at which one can obtain a higher change in the gene expression rate (~90%). See attached reference Fig. 1.

In addition, the identical injecting depth of 0.2 mm also reflects more uniform expression of 1.35 μA ± 0.3 μA in the membrane potential of oocytes (i.e., expression efficiency). See attached reference Fig. 2. On the other hand, random injecting depths in the range of 0.02-0.5 mm reflects less uniform expression 1.35 μA ± 0.65 μA in the membrane potential of oocytes. The variation of random injecting depths: 0.65 μA is more than twice bigger than the variation of injecting at the identical depth: 0.3 μA injecting at the identical depth. The identical injection depth requirement of the invention allows samples to be screened in a narrower range with a higher degree of accuracy as shown in the experiments conducted on DNA, which is applicable for mRNA.

The injection range requirement of the invention provides high expression efficiency resolves the manually injection problem of the traditional nuclei injection and cytoplasmic injection which have to be conducted manually by skilled technicians (page), last paragraph) due to various sizes of the amphibian oocytes, different thickness of their cytoplasms, and different depths of their nuclei. The identical injection depth requirement further improves the invention to obtain a higher screening accuracy via a uniform expression efficiency so provided. Such discoveries/teachings were never taught or suggested in Brown or any other prior art references that it is not inherent to inject the mRNA sample into each of a plurality of amphibian oocytes at an identical depth from a surface thereof in the range of 0.02-0.1 mm. Applicant has further determined that the change in membrane potential relates to the expression rate, whereby the membrane potential and thus the expression rate in the present invention is higher than that disclosed in the prior art.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statement were made with the knowledge that willful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-captioned application and any patent to issue thereon.

Respectfully submitted this 5 day of June, 2006